Seasonal Gonadal Changes in Two Bivalve Mollusks in Tomales Bay, California

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(Plates 58 to 60; 4 Text figures; 1 Map)

INTRODUCTION

Pododesmus cepio (Gray, 1850), a bivalve mollusk of the family Anomiidae, is native to the Pacific coast of North America and common in Tomales Bay, California. A small population of the European oyster, *Ostrea edulis* Linnaeus, 1758, is being raised experimentally at the Tomales Bay Oyster Company. The histological study of the seasonal gonadal changes of these two species, in Tomales Bay, is described below.

The study of gametogenesis in *Ostrea edulis* in Tomales Bay is desired to evaluate the reproductive adaptation of this species to a new set of ecological conditions. The inclusion of the native *Pododesmus cepio* in this study allows comparison of the gonadal development of these two species. Such a comparison aids not only in understanding the seasonal gonadal changes in both species, but also in examining the adjustment of the introduced form, *O. edulis*, to a new environment.

At the present time the oyster industry in Tomales Bay imports large numbers of oysters bred in other localities and raises them until they reach marketable size. These imported oysters, the Japanese [*Crassostrea gigas* (Thunberg, 1793)] and the American oyster [*C. virginica* (Gmelin, 1791)], do not normally reproduce in California waters. The oyster industry in California would be revolutionized if an exotic oyster, such as *Ostrea edulis*, were able to grow and reproduce in these waters.

Knowledge of the reproductive cycle in *Pododesmus cepio* is valuable not only because it offers a natural comparison to the introduced species, *Ostrea edulis*, but also because it would then be the first member of the family Anomiidae whose natural seasonal gonadal changes have been examined.

There are no references to gonadal studies of *Pododesmus cepio*. However, prior to 1953 (Fitch, 1953) *P. cepio* was considered to be the same species as a very closely related form, *P. macroschisma* (Deshayes, 1839). All available literature deals with this northern species. Kellogg (1915) described the gross morphology and ciliary currents of the mantle cavity of *Monia* (= *Pododesmus*) *macroschisma*. Frizzell (1930) published a note on the collection of a specimen of *P. macroschisma* in a *Teredo navalis* (Linnaeus, 1758) burrow from Puget Sound. Brief references were also made to *P. macroschisma* in discussions of the family Anomiidae by Atkins (1937) and Yonge (1962). There are no works on the seasonal gonadal changes in any other member of the Anomiidae.

In contrast, *Ostrea edulis* is one of the most thoroughly studied of all mollusks. Orton (1920, 1927, 1933, 1937), Cole (1936, 1941, 1942), and Korringa (1940, 1952, 1957) have studied many aspects of the reproductive cycle and sex change of the European oyster in its native waters. Loosanoff (1955) introduced *O. edulis* to New England waters in 1949 and later studied the seasonal gonadal changes of these oysters while they were adjust-

Explanation of Plate 58

Figure 1: Section of gonad of undifferentiated male *Pododesmus cepio*. Collected October 1966 ×125

Figure 2: Section of gonad of undifferentiated female *Pododesmus cepio*. Collected October 1966 ×125

Figure 3: Section of gonad of undifferentiated *Ostrea edulis*. Collected November 1966 ×125

Figure 4: Section of gonad of developing male *Pododesmus cepio*. Collected February 1967 ×500

Figure 5: Section of gonad of developing female *Pododesmus cepio*. Collected January 1967 ×125

Figure 6: Section of gonad of developing *Ostrea edulis*. Collected December 1966 ×125

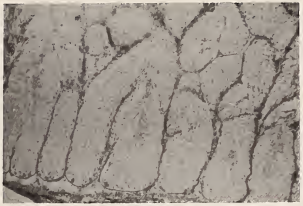


Figure 1

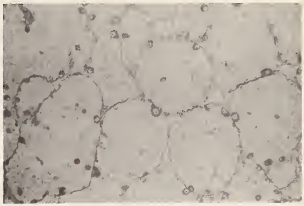
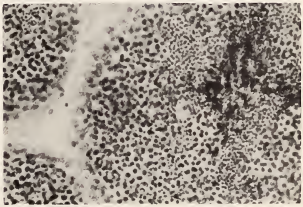


Figure 2

Figure 3



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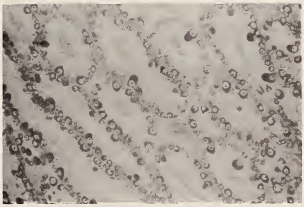


Figure 5

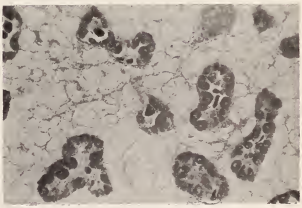


Figure 6

ing to the new environmental conditions (Loosanoff, 1962).

Three hundred European oysters were raised from larvae cultured in the U. S. Bureau of Commercial Fisheries Laboratory at Milford, Connecticut. With the assistance of Dr. Victor L. Loosanoff and the courtesy of the State of California, these oysters were imported to Tomales Bay in October 1965.

ACKNOWLEDGMENTS

I wish to thank Drs. Victor L. Loosanoff and Edmund H. Smith for their kind assistance and encouragement during this study. I also thank Mr. Carl Berg and Mr. Les Watling for aid in collecting the specimens.

METHODS AND MATERIALS

Sampling Methods

About 25 specimens of *Pododesmus cepio* were taken monthly from a natural population in White Gulch, a small cove in Tomales Bay, California (see Map, Text figure 1). Samples were collected from October 1966 to March 1968. With each collection, water temperature, salinity, and turbidity measurements were taken.

Samples of *Ostrea edulis* were collected twice a month at the beginning of the study and during the most active reproductive period (March to July), then once a month for the duration of the study. The sampling program extended from October 1966 to December 1967. As with the White Gulch samples, temperature, salinity, and turbidity were measured at each collection.

During the late spring and summer months, attempts were made to obtain larvae from the spawning oysters. To collect settling larvae, strings of cleaned oyster shells were suspended in the water around the racks in which the oysters were kept.

Laboratory Procedure

Each specimen was measured and the gonad examined externally for features, such as size and color, which might indicate its stage of development. The gonadal tissue was prepared for histological study as follows: transverse sections of the gonad, 1 to 2 mm thick, were fixed in Bouin's fluid, dehydrated in isopropyl alcohol and embedded in paraffin (melting point 52.5° C). Sections, 8μ thick, were stained with Heidenhain's iron hematoxylin and counterstained with eosin.

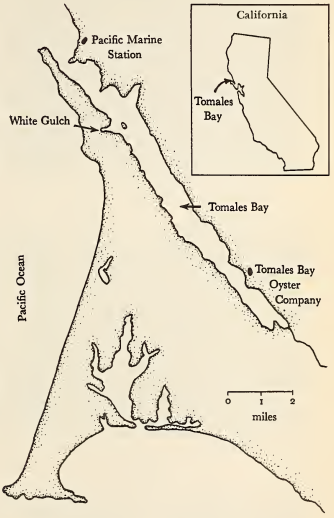


Figure 1
Map of the Study Area

CRITERIA FOR ESTABLISHING STAGES OF DEVELOPMENT

After examination of the gonadal tissue, a number from 1 to 5, representing a particular stage of development, was given to each specimen. Each of these stages represented a naturally occurring and characteristic change in the gonads of the animals during the entire reproductive cycle.

Stage I

This stage represents the resting or indifferent phase through which *Ostrea edulis* and *Pododesmus cepio* pass

following the activity of the previous reproductive period (Plate 58, Figures 1, 2, and 3). In *O. edulis,* the gonadal tissue surrounding the digestive gland is reduced to a thin, translucent layer less than 1 mm thick. The fine tubules connecting the follicles to the genital ducts can be seen through this layer. In *P. cepio,* the gonad forms a layer of tissue partially covering the stomach and continues anteriorly as an irregular tube attached to the byssus gland. During this period the gonad is clear, and the delicate genital tubules can be seen beneath the surface epithelium. The gonad of *P. cepio* is usually 2 - 3 mm thick at this time.

In both species the follicles are separated by connective tissue, often forming isolated pockets of follicular tissue. Usually the follicles are small and completely void of sex cells. However, primary germ cells are sometimes present and are attached to the follicular wall. The gonads of both species are colorless at this time; it is difficult to determine their sex.

Stage II

Early gametogenesis in *Ostrea edulis* and *Pododesmus cepio* is characterized by the development of primary and secondary oogonia, spermatogonia, and, in some advanced cases, oocytes and spermatids (Plate 58, Figures 4, 5, and 6). The connective tissue surrounding the follicles supplies nutrient material (glycogen) for the developing cells (Loosanoff, 1942). As the sex cells proliferate, the follicles slowly enlarge and become more numerous. During early development all stages of gametogenesis can usually be found in one individual. *Ostrea edulis* often has male and female sex cells in various stages of gametogenesis in the same or adjacent follicles. In general, however, mature gametes are not present at this time.

Externally the gonads become opaque as the developing cells fill the follicles. At this time the gonad of the female *Pododesmus cepio* has a faint red-orange color which becomes more brilliant as the animal ripens. Throughout their development, the gonads of the male have a creamy white color, similar to that of *Ostrea edulis.*

The later stages of gametogenesis are characterized by the rapid increase in numbers of mature eggs and sperm. In *Ostrea edulis,* ripe eggs interspersed with oocytes line the follicles. In males and hermaphroditic individuals, the spermatids and spermatozoa fill the lumen of the follicle. In this species the sperm characteristically form spermballs with the tails projecting outwards as in Plate 59, Figure 9.

As the gonads of *Pododesmus cepio* ripen, eggs rapidly fill the follicle, with a corresponding decrease in the number of cells in earlier stages of oogenesis. Male follicles typically have primary stages of spermatogenesis in the outer portion of the follicle, with more mature sex cells occupying the lumen. In *P. cepio,* the sperm are arranged in branching networks with their tails aligned toward the center of the follicle.

Externally the gonads of both species become firmer and larger than during earlier stages. The color also becomes brighter, especially in female *Pododesmus cepio.*

Stage III

When fully ripe, both species possess large, characteristically swollen follicles containing mature gametes (Plate 59, Figures 7, 8, and 9). Developing stages of gametogenesis are seldom present at this time. Ripe ova and spermatozoa are commonly found in the genital ducts awaiting discharge. Mature eggs in *Pododesmus cepio* often have a highly compressed appearance. The eggs are 60 to 75μ in size at this time. In *Ostrea edulis,* the eggs have a diameter of 85 to 90μ but do not appear compressed.

At this time the gonad of *Pododesmus cepio* attains its largest size, sometimes a centimeter or more in diameter. *Ostrea edulis,* however, never develops a thick gonadal layer. In Tomales Bay, as in Boothbay Harbor, Maine (Loosanoff, 1962), the gonad of *O. edulis* is rarely more than $1\frac{1}{4}$ mm thick. The ripe condition in the European oyster is obvious when the gonad is punctured, allowing the tightly packed gametes to be released.

Explanation of Plate 59

Figure 7: Section of gonad of ripe male *Pododesmus cepio.* Collected June 1967 ×125

Figure 8: Section of gonad of ripe female *Pododesmus cepio.* Collected June 1967 ×125

Figure 9: Section of gonad of ripe *Ostrea edulis.* Collected May 1967 ×125

Figure 10: Section of gonad of spawned male *Pododesmus cepio.* Collected August 1967 ×125

Figure 11: Section of gonad of spawned female *Pododesmus cepio.* Collected August 1967 ×125

Figure 12: Section of gonad of spawned *Ostrea edulis.* Collected August 1967 ×125

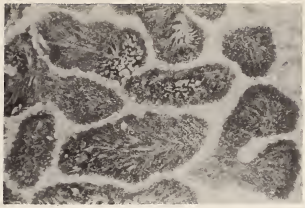


Figure 7

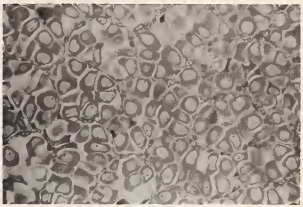
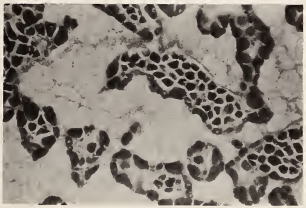


Figure 8



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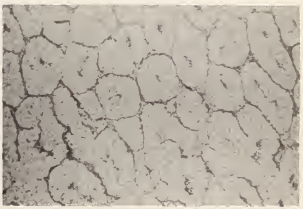

Figure 10

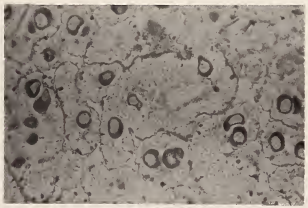

Figure 11

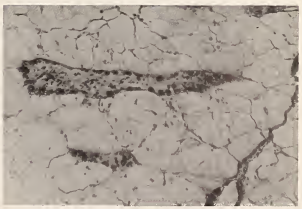

Figure 12

Stage IV

Examination of recently spawned specimens of *Pododesmus cepio* and *Ostrea edulis* reveals large empty follicles (Plate 59, Figures 10, 11, and 12). Although a few unspawned gametes are often visible in the lumina of the follicles, no developing cells are present. In many cases, partially spawned animals will have empty follicles on the periphery of the gonad but fully ripe follicles closer to the digestive gland. Specimens of *O. edulis* commonly have cells of the next sexual phase undergoing gametogenesis in the evacuated follicles immediately following spawning. For example, a female phase may be completed early in the summer with a male phase beginning soon after the spawning of the eggs.

The appearance of the gonads following spawning is dramatically different from the previous ripe condition. The gonads are greatly reduced in size, no longer firm, and usually void of sex cells in areas adjacent to the genital ducts. This shrunken condition is especially obvious in *Pododesmus cepio*.

Stage V

Ostrea edulis and *Pododesmus cepio* pass through a period of resorption following spawning, in which any remaining sex cells are absorbed by phagocytes (Plate 60, Figure 13). During this time the follicles of both species are small; the lumina are filled with phagocytes and fragments of partially resorbed gametes. Resorption is a slow process and often extends over several months' time, thereby overlapping with the development of new gametes.

GAMETOGENESIS

It is appropriate to begin the description of gametogenesis in *Pododesmus cepio* and *Ostrea edulis* with the inactive period (Stage I) following spawning and resorption. In Tomales Bay this period occurred during October and November, in both 1966 and 1967. The water temperature was 16° C over the *O. edulis* population and approximately 13° C in White Gulch (Text figures 2 and 3). The gonads of both species were typically in the Stage I condition, undifferentiated and empty. During this stage the oyster usually had some primary germ cells attached to the follicular wall, while *P. cepio* was completely barren. The duration of this indifferent period was similar for *P. cepio* and the oyster (Tables 1 and 2).

Early gametogenesis (Stage II) began late in November 1966 and continued until April and early May 1967.

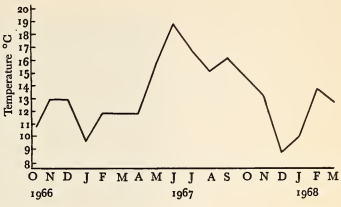

Figure 2

Water Temperature at White Gulch, Tomales Bay, California, from October 1966 to March 1968

Although the water temperature in both study areas dropped to 12.5° C, each species had begun to show gonadal differentiation by early December 1966. The European oyster developed more rapidly than did *Pododesmus cepio*. Ripe *Ostrea edulis* were found as early as December 1966, while a significant number of ripe *P. cepio* was not found until April 1967. All stages of spermatogenesis and oogenesis were found during this active period. In both species, individuals were found lagging behind or developing more rapidly than the rest of the specimens of the sample.

As the water warmed from a low of 9° C in January to 12 - 13° C in March 1967, both species continued to

Figure 3

Water Temperature at Tomales Bay Oyster Company, California, from October 1966 to January 1968

Table 1

Numbers of *Pododesmus cepio* in different stages of gonad development recorded at each collection; October 1966 to March 1968

Stage	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March
	1966								1967							1968		
I.	7		4	1	3	1					6	2	10	12	10			
II.	1	12	11	14	15	29	30	34	3	1	1		3	4	10	31	33	15
III.		3				1	4	1	15	8	1		4	10	3	3		
IV.									10	22	22		10	4				
V.	2								1	2	16	5	11	12	12	3	2	

Note: The approximate duration of each stage is indicated by the blocks surrounding the numbers in the table above.

Table 2

Numbers of *Ostrea edulis* in different stages of gonad development recorded at each collection; October 1966 to December 1967

Stage	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December
	1966								1967						
I.	10	9	1										4	8	5
II.	2	20	26	10	13	11	17	4	5					1	1
III.	1		1	3	2	3	11	12	17	14	1	2	4	5	2
IV.						1	1	4	8	3	6	3		1	
V.	1						1			1	1	4	1	1	1

Note: The approximate duration of each stage is indicated by the blocks surrounding the numbers in the table above.

Explanation of Plate 60

Figure 13: Section of gonad of resorbing female *Pododesmus cepio*. Collected November 1967 ×125

Figure 14: Section of gonad of hermaphroditic *Pododesmus cepio*. Collected November 1967 ×125